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EFFECT OF GROWTH-SUBSTANCES ON THE FREE AMINO-ACID CONTENT OF LENTIL SHOOTS IN CASE OF WATER DEFICIENCY

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Abstract

The free proline is not accumulated in an extremely high degree, as a result of a strong water deficiency, in the leaves of every plant species. From among 80 mono- and dicotyledonous herbaceous plant species belonging to fifteen families fifteen species of plants are storing proline but in a comparatively lower degree in case of water deficiency.

We have established which is — at time of a comparatively equal but strong water deficiency — the proline-concentration level in case of attainment of which we may speak of a "plant species of proline-accumulating type". This proline quantity is 10 mg/g dry matter. On the basis of that, the majority of the 80 plant species investigated, belonging mainly to the cultivated plants, may be regarded as proline-accumulating ones.

Studying the part of the free proline accumulated during water deficency, in the course of the developing or ceasing drought, it is not the same whether we are examinig a plant of proline-accumulating type, or not, because the ways of the free *aminr-acid* metabolism and enzyme systems of the non-proline-accumulating species differ from those of the proline-accumulating plants probably considerably.

We have established that in the course of the development of a strong water deficiency in the "proline-type" lentil shoots, the growth-substances applied (IAA, FAP, GA_3) exerted their influence on the water content of shoots and increased their free total amino-acid and proline content considerably.

The greatest effect was achieved by the 10 mg/l solution of IAA that increased the free total amino-acid content of lentil shoots 200 p.c., and their proline content 58 p.c., as compared with the water-treated control.

It can be concluded from the results of our investigations concerning the water balance that the IAA-treatment may be advantageous in regard of the drought-resistance of lentil shoots. This treatment provided, namely, the largest bound water content, as well.

In the course of our experiments, after IAA, the 20 mg/l FAP-treatment resulted in the largest increase in proline concentration; the 50 mg-l GA_3- treatment, however, increased proline-accumulation but in a lesser degree, and it had no considerable effect on the water balance, either.

In the course of our experiments, after IAA, the 20 mg-l FAP-treatment resulted in the largest increase in proline concentration; the 50 mg/l GA -treatment, however, increased proline-accumulation but in a lesser degree, and it had no considerable effect on the water balance, either.

We have demonstrated the physical, chemical, and physiological factors on the basis of which — at the proline-accumulating types — the high proline level is advantageous in regard of tolerating water deficiency.

Introduction

We established in the course of our investigations lasting for several years on the subject of plants grown in the field and in culture-vessels (PÁLFI, 1968a, 1969, 1971; PÁLFI et al., 1973, 1974) that in the majority of mezophytes (herbaceous), in case of strong water deficiency, the total amino-acid and first of all proline content of leaves significantly increased. A similar conclusion was drawn by BARNETT and NAYLOR (1966), BOKAREV and IVANOVA (1971), LEWITT (1972), VALLEE (1973), and PERDRIZET (1974).

According to the investigations of Stewart et al. (1966), that is a common phenomenon in the circles of the various plant families. KUDREV (1970), however, is taking the view that the level of proline accumulation is quite different according to plant species. In his opinion, if e.g. in bean proline achieves a medium level, a further synthesis comes to a stop, because of the end-product inhibition of enzymes.

Some have striven to clear up the path ways of proline production by applying inhibitors of growth, glycolysis, and respiration, respectively.

BRITIKOV and LINSKENS (1970) applied inhibitors of glycolysis (NaF and Na-azide) but they performed their experiments mainly on spinach, resp. maize and these plants, as we ascertained (PÁLFI, 1971; PÁLFI et al., 1973), don't store up a very large quantity of proline in the time of the greatest water deficiency, either.

According to STEWART et al. (1966), arsenate, iodine-acetate, and iodine-acetamide probably inhibit glycolysis, resp. the formation of alpha-ketoglutarate. Therefore, there cannot be produced any glutamic acid, the starting compound of proline synthesis.

In our earlier investigations (PÁLFI, 1968b), kinetine and 2.4—D did not prevent water-deficient plants from accumulating proline resp. a large amount of free amino acid, and proline synthesis was not brought to an end by maleic hydrazide and chloramphenicol, either. Only 2.4-dinitrophenol was effective in this respect — by uncoupling the oxidative phosphorilation. And as proline synthesis demands ATP, its accumulation did not take place.

In our present experiment we have first of all endeavoured to clarify if every species of herbaceous mesophytes is storing proline in an extremely large measure in its leaves, in case of strong water deficiency achieving 2—4 per cent of dry matter.

We are investigating, as well, which is the proline concentration after achieving or exceeding of which we may speak of an extraordinary proline accumulation; further on, if a high-level proline accumulation is advantageous to plants in regard of tolerating water deficiency.

Finally, we have studied whether some growth-regulating substances: indole-3--acetic acid, furfurylaminopurine, gibberellic acid increase or decrease the total amino-acid or proline synthesis and accumulation during water deficiency. We are appreciating, too, the influence of growth-substances on water balance, on the quantity of dry matter and soluble total protein.

The effect of growth-stimulating substances on the water balance of plants was investigated recently (TRUNOVA, 1968; MIZRAHI et al., 1970; DERBYSHIRE, 1971; ITAI and VAADIA, 1971; TUCKER and MANSFIELD, 1971; LOESCHER and NEVINS, 1973).

BADANOVA and LEVINA (1970) established in barley that drought-resistance was increased somewhat by CCC, and decreased by GA_3.

ZADANTSEV and PIKUS (1973) found so that in case of wheat CCC was promoting to tolerate water deficiency and high temperature. The changes in free amino acids were, however, outside the scope of their investigations.

Materials and Methods

For clearing up the type of amino-acid metabolism during drought respectively for classifying the plants, we have mostly collected the shoots or leaves of plants grown in field. The shoots of entil *(Lens culinaris)* were grown in culture-vessels by soil culture.

The solutions of indolacetic acid (IAA), kinetine (furfurilaminopurin—FAP), and gibberellic acid (GA_3) were prepared from the chemicals of the firm E. Merck, Darmstadt.

For analysing amino acids, we have started from 200 mg leaves dried up till getting steady weight at 60° C and pulverized. The qualitative analysis of amino acids was carried out in a part of experiments by means of ascending paper chromatography. We have worked by developing with retarded cooling, the solvent being butanol-acetic acid-water (3:1:1), and a tproline detections phenol-water (4:1).

We had already published the method of measuring free proline and total amino acid by eluation (PÁLFI, 1968a, 1971).

At measuring total amino acid, we have eluted with methanol, and at proline determinations we have done that with water-saturated phenol. At total amino-acid determinations, the calibration curve was constructed from the measurements of series of different concentration of the so-called "universal standard mixture" composed by us from sixteen amino acids. The composition of standard, in qualitative as well as quantitative respects, was similar to the pattern of plant extracts.

The soluble total protein was determined according to LOWRY et al. (1951), and tris-buffer was used for preparing extracts (pH 7.5).

At the automatic amino-acid analyser "BIOCAL BC 200" buffers of 3.25, 4.25, and 5.28 pH-value were applied.

The mean error in the average results of the quantitative analyses carried out in 3 to ,5 repetitions, that is their standard deviation was less than ±5 to 7 per cent.

Experimental results

1) "Proline" and "non-proline" types of the amino-acid accumulation in the course of water loss.

In the course of our experiments we have taken into consideration ourearlier establishment (PÁLFI, 1973, PÁLFI et al. 1974) that in the time of the so-called "live-wilting", i.e., the gradual water loss of the detached shoots and leaves during being lighted, there are taking place the same biochemical processes as in case of intact plants. The difference is mainly that for the formation of a strong water deficiency in the native plants grown in the field a four to six weeks long time without precipitation resp. irrigation being necessary. The same high-level water deficiency in detached leaves is attainable already in two to five days. During that time, the total amino-acid content and proline-level are increasing to the highest degree, the same as in the leaves of native plants.

With the method, elaborated by us, of "live-wilting" the leaves and shoots detached, we have investigated the free amino-acid, resp. proline concentration of 80 plant species of 15 families, in case of an optimum water supply and in that of a developing, strong water deficiency.

"Live-wilting" took place for four to five days, under permanent lighting (5000 Lux), while we achieved a 40 to 45 per cent water deficiency in the (green) plant parts detached (in the percentage of the fresh matter in the varieties irrigated). From the results obtained, we are giving some data in Table 1.

It turns out from the data of Table 1 that, at any mono- and dicotyledons, the total amino-acid resp. proline content is significantly increased. While anyway the quantity of total amino acid in all the species has increased to be three-six-times as

Table 1. Free proline and total amino-acid content of detached leaves and shoots of mono- and dicotyledonous soft-stalk plants, as a result of a water loss for 4 to 5 daysat 25° C, besides 60 per cent relative vapour content and illuminated permanently with 5000 Lux, in the prebloom period of development. The proline content of the leaves of plants grown under optimum water conditions was below 0.4 mg/g dry matter, and their total amino-acid content below 20.0 mg. The species were arranged in families and separated with line. (The average deviation of the analyses carried out in three repetitions was below ±7 per cent.)

Plants	Proline	Total amino acid	Plants	Proline	Total amino acid
	mg/g dry matter			mg/g dry matter	
Sinapis alba	24.8	65.4	*Helianthus annuus*	21.4	47.3
Raphanus sativus	26.4	63.2	*Artemisia vulgaris*	23.7	52.8
Brassica napus	25.9	57.5	*Matricaria chamomilla*	22.1	56.8
Brassica oleracea	43.7	110.2	*Lactuca sativa*	3.0	49.6
Solanum tuberosum	29.6	82.5	*Taraxacum offici.*	4.2	45.7
Capsicum annuum	35.2	53.7	*Pisum sativum*	33.1	106.3
Nicotiana tabacum	28.5	75.3	*Lens culinaris*	22.1	70.4
Sol. lycopersicum	22.3	63.2	*Medicago sativa*	20.3	104.6
Hyoscyamus niger	26.5	68.4	*Trifolium repens*	21.4	89.6
Sol. laciniatum	32.6	72.1	*Phaseolus vulg.*	8.6	53.4
Triticum aestivum	25.4	82.4	*Chenopodium album*	4.7	70.1
Hordeum vulgare	18.6	59.9	*Spinacia oleracea*	4.2	72.5
Secale cereale	17.9	64.5	*Beta vulgaris*	3.8	67.5
Avena sativa	21.3	54.6	*Rumex scutatus*	3.3	54.7
Sorghum vulgare	20.4	58.2	*Anethum graveol.*	18.2	56.6
Cynodon dactylon	25.7	60.1	*Rubus caesius*	21.8	54.0
Festuca pratensis	22.8	63.7	*Allium cepa*	4.2	47.6
Bromus arvensis	26.4	52.8	*Allium sativum*	3.6	52.9
Poa pratensis	23.6	54.5	*Cucurbita pepo*	3.4	53.2
Lolium perenne	31.4	63.6	*Cucimis sativus*	3.0	44.5
Lolium aristatum	28.5	71.0	*Cucumis melo*	2.9	47.6
Zea mays	5.1	53.6	*Colocynthis citrullus*	3.5	56.2
Vitis vinifera	7.9	45.6	*Papaver somniferum*	4.0	41.7

the (non-water-deficient) control, the proline concentration has given quite striking differences in the various species. The increase in proline content is, therefore, changing from sixfold *(Cucumis melo)* till 135-fold one *(Capsicum annuum)*. It follows from the data that it is to be decided, which level is to be regarded as an extraordinary increase in proline, respectively as a proline-type amino-acid metabolism in the course of water deficiency.

On the basis of our several experimental data, we consider as a proline-accumulating type during water-deficiency the herbaceous mesophytous plant species the young but full-developed green leaves of which native plant, or the shoots detached and leaves isolated of which, in the course of their "live-wilting", resp. during losing gradually 40 to 50 per cent of their water content at being illuminated, accumulate at least 10 mg free proline, as related to 1 g dry matter. (The determination is to be performed in the phase of budding, resp. blooming, at a temperature and vapour content that correspond to the water loss becoming gradually ısronger, resp. to the „live wilting" in case of any plant species.)

It turns out from Table 1, as well, that not every plant species may be regarded as a proline-accumulating type, in case of water-stress, as we have obtained in 15 plant species of the Table a proline quantity that is considerably below 10 mg. We have, therefore, to abstain from the fact of a general, extraordinary proline accumulation as a result of a strong water deficiency in plants.

It is anyhow to be noted that in the Table 34 plant species of proline-accumulating type are not published from among the species investigated so far. Taking all this into consideration, we may establish that, on the basis of our investigations, the majority of plant species belong to the proline accumulating type.

In the course of our experiments, we laid the main stress upon screening the proline-accumulating species surely. That is not an easy task because if we hadn't achieved a 10-mg level at some species in case of a strong water deficiency, either, then we often had to repeat the experiment, under the conditions of a water loss of quite different degree and rate. In the course of our careful investigations carried on for several years, we fell nevertheless into error not only on one occasion. At any rate, in Table I, we are publishing the highest values of proline content from among those of non-proline-type species measure during the repeated investigations.

From our results obtained, we may already draw the conclusion that before beginning to investigate the free amino-acid spectrums and protein synthesis developing as a result of water deficiency, we have to establish the type of accumulation at every species. The experiments may only be continued after taking into consideration these data because the reaction ways and regulative mechanisms of the proline accumulation of extraordinary size are differing from those in plants of non-proline type. That was established by TYANKOVA (1969), as well.

In the course of our experiments, we established undoubtedly and several times that lentil (and 70 to 80 per cent of the species investigated of the Leguminosae family) belong surely to a type accumulating proline in case of a strong water deficiency. This is the reason of choosing lentil for the aim of our present research work.

2) The effect of growth-substances upon accumulating free amino acids in the course of water loss

From the isolated lentil shoots we weighed out equal quantities and, after eluting them carefully, we evaporated the water adhering to the surface. Then we had the water, resp. growth-substances weighed in an exact quantity into the beakers, absorbed through the stalk and at being illuminated, for 24 hours (Fig. 1). In the meantime, in order to avoid infections, we weighed and changed the fluids of tumblers in six hours. At being placed into the solution in beakers, the shoots and leaves were somewhat wilted. The full turgor, resp. normal water supply was regained by any sample already in two to three hours after being steeped in the fluids.

After treating them for a day, the weight of lentil shoots was established in a water-saturated state. Then followed the "live-wilting" for 48 hours, by illuminating

Fig. 1. Treatment of 4-week detached lentil shoots (*Lens culinaris* L.), grown in culture, with the solutions of growth-regulating substances, for 24 hours, illuminated with 5000 Lux. Varieties: "AB" = tap water; "C" = IAA 10 mg/l; "D" = FAP, 20 mg/1; "E" = GA_3, 50 mg/l.

the samples (5000 Lux) and engendering a gradual water loss, during which the 60 to 70 per cent relative vapour content of air was provided by means of a foil-cover.

After "live-wilting" for two days, we have weighed the samples (withering weight), then we took samples for determining the soluble total protein. Then, fixing the single varieties, we weighed their dry matter, as well. The data are given in Table 2.

It turned out from Table 2 that the lentil shoot of 100.0 g fresh weight absorbed a considerable fluid quantity during the 24 hours. Most water was absorbed by the sample placed into the FAP solution, and least of that by the sample treatment by GA_3. But in the water quantities absorbed there wasn't found any considerable difference. The water quantity stored in the plant is proving that a water saturation was produced and the fixation of water of different quantities was made possible

Table 2. The fresh weight of the lentil shoots pre-raised under identical conditions and detached (100.0 g), the amount of water absorbed during 24 hours and the weight and dry matter after being wilted in light during the following 48 hours, as a result of water, IAA, FAP, and GA_3. The starting amount of solutions is 150.0 ml. The average deviation is below ±5 per cent.

Treatments of lentil shoots (The total weight of one sample is 100.0 g fresh matter)	Quantity of solution absorbed during treatment ml	Increase in weight of 100 g shoot after treatment g	Weight of shoots afrer the live-wilting for 48 hours g	Final (dry) weight of 100 g shoot g
Shoots fixed immediately after detachment (fresh, control)	—	—	—	21.8
Shoots fixed after a water treatment for 24 ours following detachment (control)	76.0	12.1	—	21.3
Held in water for 24 hours and "live-wilted" for two days	76.8	12.4	35.6	20.2
Held in the solution of IAA 10 mg/l for 24 hours and „live-wilted for two" days	78.2	16.0	37.1	21.1
Held in the solution of FAP 20 mg/1 for 24 hours and "live-wilted" for two days	80.3	15.8	34.0	20.6
Held in the solution of GA_3 50 mg/l for 24 hours and "live-wilted" for two days	75.5	14.5	35.9	21.0

by the growth-substances applied. The water content of lentil shoots was most increasing as a result of being treated by the IAA solution (16.0). The bending of shoots (Fig. 1) is, therefore, not a consequence of water deficiency, resp. of wilting. It was also established that the bend of lentil shoots intensifies owing to the concentration of IAA solutions from 10 to 50 mg/l.

Fig. 2. Post-treatment wilting of lentil shoots detached, for 48 hours, under 5000 Lux illumination, below foil cover.

Varieties are the same as in Fig. 1, except for the watered control that was already fixed.

The water content of shoots was increased by being treated with FAP and GA_3 solutions, as well, as related to the control shoots treated with tap water.

It can be established from Table 2, as well, that the absorption treatment with IAA solution promoted not only the absorption of the largest water quantity but, after "live-wilting" for two days, the strongly fixed water content, too, remained the largest in case of this variety (37.1), although the difference between varieties is not considerable. In the dry matter of shoots we have not obtained any important differences, either, but in case of the growth-substances the final dry-weight is generally smaller than that of controls, that is to say, than that of the freshly fixed one and the variety administered with water for 24 hours.

From among the results of the qualitative amino-acid analysis, we are demonstrating an ascending one-dimension paper chromatogram (Fig. 3).

Taking into consideration that in the chromatogram seen in Fig. 3 we let run a standard mixture of the amino acid of known composition and concentration (stripe A), as well, we can draw approximative quantitative conclusions, too. We can establish that the total amino-acid content, as a result of "live-wilting" for two days, in all the four varieties, has increased to at least a double one (stripe CDEF), as compared with the control (stripe B). Most total amino acid, resp. proline, accumulated during being treated by IAA-solution (stripe F).

From Fig. 3 it can be established, too, that — compared with the samples fixed immediately after being cut (stripe B, fixed freshly) — about 15 to 20 per cent amino-acid increase was realized during the water imbibation lasting for 24 hours (stripe G).

In the course of this water imbibation of the lentil shoots detached, taking place in a bunch for 24 hours, particularly the quantity of leucine, phenylalanine, valine and methionine, pipecolic acid, asparagine and cysteine increased.

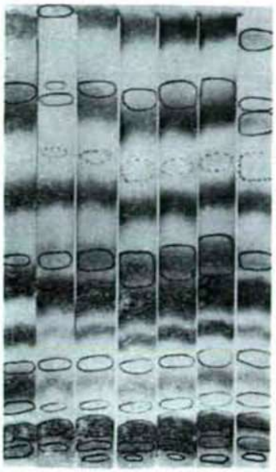

Fig. 3. Change in the free amino-acid composition in the lentil shoots detached, as a result of a 24-hour treatment and a "live-wilted" in light, for 48 hours. Control: shoots fixed immediately after being detached and the variety held in water. Ninhydrinic colour reaction, fixed with copper nitrate.[1]

"A" Comparing standard, its total amino-acid content being 60 μg; "B" = fixed immediately after being detached (fresh control); "C" = heldin water and "live-wilted" for two days (with gradual water loss); "D" = treated by FAP-solution and "live-wilted" for two days; "E" = treated by GA_3—solution and "live wilted" for two days; "F" = treated by IAA-solution and "live-wilted" for two days; "G" = fixed after being steeped in water for 24 hours (control).

It was determined the quantity of the free total amino acid and proline, as well as that of the soluble total protein, too. Results are published in Table 3.

From Table 3 it is to be established that in the course of the water-losing "live-wilting" lasting for two days the proline content accumulated to the highest degree as compared with the control supplied with water to the optimum degree. The highest proline level was however induced, as established at Fig. 3, as well, by the administration with IAA-solution. It is proved by the data, too, that the lentil plant belongs, in case of drought, really to the proline-accumulating type as the free proline content of all the four kinds of the "live-wilting" varieties (with water deficiency) has exceeded the 10 mg/g dry-matter level. It is to be noted, as well, that in case of lentils precultivated under optimum conditions in the field, in the course of losing water at "live-wilting", a much higher proline content can be achieved than the quantities included in Table 3.

In regard of the total free amino acid, as well, the highest value was given by the treatment by IAA-solution, as it has increased to 300 per cent of the fresh control. Apart from that, the total amino-acid content of every water-losing variety surpasses the double amount of the control considerably. The proline, resp. total amino-acid content was increased, to a greater or lesser degree, not only by being treated by IAA-but also by FAP and GA_3-solutions, as compared with the water-treated control that was wilted for two days.

Table 3. Free proline, total amino-acid and soluble total protein content of the lentil shoots precultivated under identical conditions and detached (100.0 g fresh matter), as a result of stepping treatment in an agent for 24 hours and water-losing "live-wilted" at light for 48 hours. (The average deviation is below ±7 per cent. The control is a water-treated variety.)

Treatment of the lentil shoots detached: 100.0 g fresh matter	Free proline	Total amino acid including proline	Soluble total protein: mg/g living matter
	mg/g dry matter		
Shoots fixed immediately after detachment fresh, control	0.32	14.9	18.8
Fixed after water treatment for 24 hours following detachment (control)	0.31	17.3	18.5
Held in water for 24 hours and "live-wilted" for two days	11.6	34.8	18.4
Held in the solution of IAA 10 mg/l for 24 hours and "live-wilted" for two days	18.4	49.6	18.0
Held in the solution of FAP 20 mg/l for 24 hours and "live-wilted" for two days	15.9	40.9	18.4
Held in the solution of GA_3 50 mg/l for 24 hours and "live-wilted" for two days	15.3	44.1	18.6

Although the highest value in regard of the soluble total protein content was given by the sample fixed immediately after being cut, there is no significant difference between the single samples as the differences fall under the error limit of average results.

We have checked our data of amino-acid analysis by means of an automatic amino-acid analyser of type "BIOCAL BC 200", as well. Four curves of the results are shown in Fig. 4.

In the course of this analysis, too, the free proline content of the sample treated by IAA-solution increased to the highest degree as compared with the contro- that was held in water. We established, too, that the proline content of lentil shoots was increased by the FAP- and GA_3-solution treatment, as well, and that in the figure from among all the amino acids the quantitative changes in proline are the most significant.

3) The significance of the high proline accumulation takingplace- in the course of losing water in regard of tolerating water deficiency

Further on, we have wanted to establish the advantages of a higher proline accumulation in tolerating water deficiency in case of the single plant species.

At the proline-type plants we have established that a high proline concentration is advantageous and we are supporting that as follows:

1) The hygroscopic nature of proline and its water-fixing capacity is the highest among all the protein-forming amino acids. Its water-solubility is standing in the

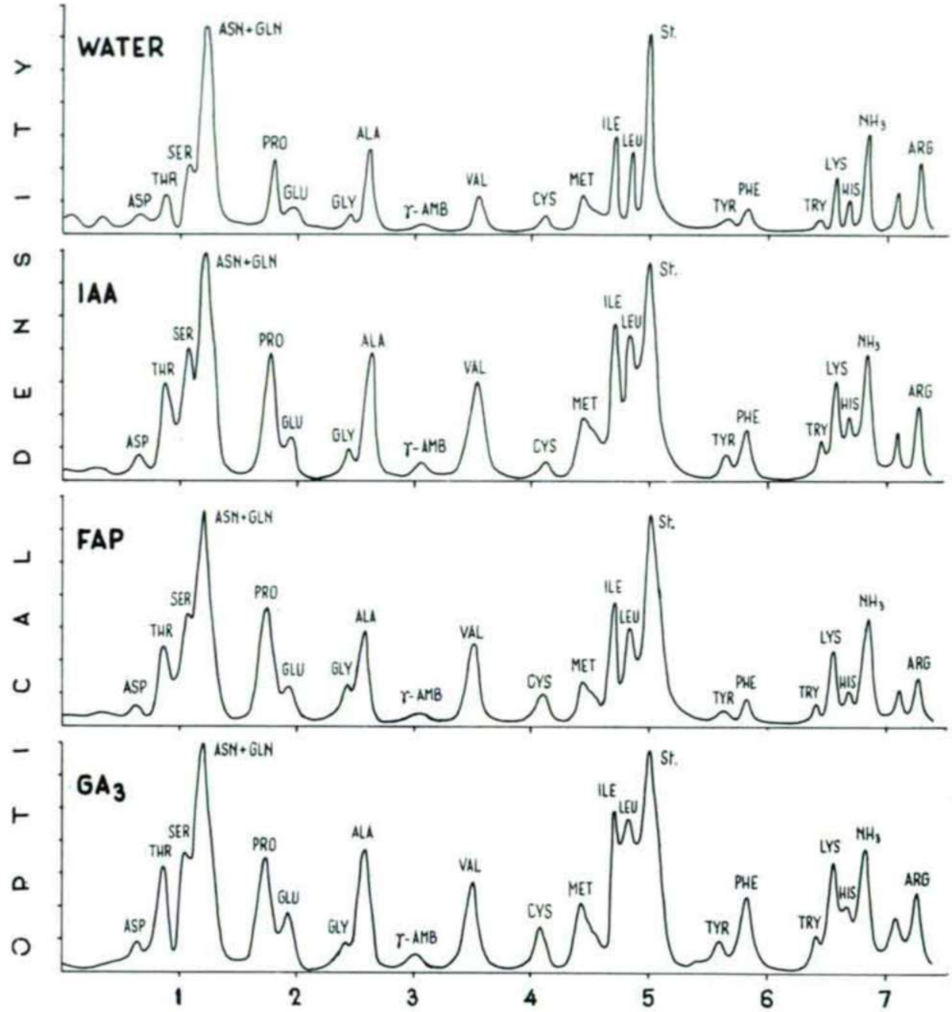

Fig. 4. Free amino-acid content of water-deficient lentil shoots, "live-wilted" for 48 hours after being treated, measured with an automatic amino-acid analyser of "BIOCAL BC 200"-type. Curves downwards: Water-, IAA-, FAP-, and GA_3-treated samples. (Average deviation being below ± 5 per cent.)

first place, too: at syntheses and transaminations, the most frequently involved glutamic acid is soluble 192-times, and aspartic acid 300-times more poorly in water than proline does. It may therefore be in the tissues of plants in a dissolved, active state, even in the more and more decreasing water (physical advantage).

2) During hydrolysis the free amino acids with 6 n HCl, for 24 hours, at 110° C, under pressure — in the presence of KNO_3 as oxidizer — every protein-forming

amino acid was decomposed, except for proline. The proline-stability is extremely high (chemical advantage).

3) Proline, during its being formed from glutamic acid, is storing reducing energy coming from photosynthesis and that gets released after the water deficiency being ceased and proline reverting to glutamic acid again. Owing to this redox property, proline has a respiration-influencing role, as well.

4) The high concentration of free proline in the tissue — as compared with the other amino acids — is favourable to growth because it is less toxic, as we have proved that with germinating experiments carried out under sterile conditions and with an oat coleoptil test (physiological advantages).

On the basis of our results we can establish that a high-degree proline accumulation is favourable to plants in the period of tolerating drought. Therefore, in case of proline-type species, we can predict the beginning of water deficiency by demonstrating the proline quantity accumulated during the drought (PÁLFI and JUHÁSZ, 1971). The method can be applied to selecting the drought-resistent plant species, as well (PÁLFI et al., 1973, SINGH et al., 1972). That is confirmed by the experimental results of *Waldren* and *Teare* (1974), too.

Evaluation of results

We have cleared in the first part of our experiments if each of the herbaceous mesophytous plant species, mono- and dicotyledons, accumulates proline to a great extent in case of water deficiency. On the basis of the extent of proline accumulation, the plants were classified into two types. If the proline concentration that is developing in the leaves of the plant in light gradually in case of a strong water deficiency reaches or surpasses the 10 mg/g dry matter quantity, the species is, in our opinion, a proline-accumulating one and the emerging picture of amino acid is of "proline type".

From among the 80 species investigated, only 15 did not prove to be proline accumulating-ones. The amino-acid picture of the majority of plants investigated is, therefore, of "proline type". The proline content of bean shoots often approximated the 10 mg/g quantity in case of water deficiency but it did not surpass it. We consider it, therefore, as an intermediate type.

It was established also by KUDREV (1970) that, in case of bean, the proline accumulated exerts an effect on the enzymes taking part in the synthesis by means of end-product inhibition. Its storing comes therefore to a stop at a certain level of concentration.

We cannot consider as final, therefore, the establishment of STEWART et al. (1966) that a considerable accumulation of proline in case of water deficiency is a common phenomenon at plants. It is true that the authors drew that inference on the basis of their results obtained only at eight plant species investigated. STEWART (1971, 1972) examined also the biochemical ways of transformation of the proline of exogenous origin in bean plants, in the dark. According to us, the behaviour of bean is not characteristic of proline-type plants in this regard, either.

In case of lentils, on the other hand, we may regard as advantageous to hold amino acid, resp. proline in store as this species belongs to the proline-accumulating type.

On the basis of water balance, we may consider as proved that the treatment with IAA, respectively the increased amino-acid content, as well, can be regarded as advantageous for tolerating drought. If we investigate, namely, the data obtained in case of lentils concerning water balance, then it turns out that the difference at the various treatments is not considerable as regards water uptake and water loss but the water content bound in the shoots has grown the most as a result of IAA-treatment. That variety contained most water after withering two days long, as well. It follows from that the bend experienced at lentil shoots is not a consequence of the decrease in turgor but it is epinasty.

The cold-resistance of plants was investigated by TRUNOVA (1968). According to her, as well, the water flow out of the cells is reduced by IAA-treatment and with that the water content of cells is increased. In respect of cold-resistance, this phenomenon is anyway not advantageous because if water remains inside the cell and freezes there then the plant perishes.

IAA-experiments were carried out by KUDREV and TYANKOVA (1966), as well. They were, however, examining if after the water deficiency that the plants had survived, the quick rehydration and the normalization of the amino-acid metabolism were advanced by IAA.

KUDREV and TYANKOVA experimented with full-grown wheat of proline-accumulating type and they applied 100 mg/l IAA-concentration. In their opinion, IAA undeniably promoted the restoration of water balance and a quick decrease in the high concentration of free amino-acid and proline, respectively.

Bin and STANLEY (1972) examined the effect of IAA-solution on the water balance in the stalk-segments of peas. According to them, the water flow was accelerated by the 10—30 μMol concentration of IAA in both directions, that is to say, in the tissues both inwards and outwards (and even inside of the tissues, as well). In the authors opinion, the IAA-solution exerts its effect on the permeability of membrane systems.

LIVNE and GRAZIANI (1972) established concerning kinetine (FAP) that it filled an important part in regulating the water-permeability of leaf-tissues. In the course of our experiments, the treatment with FAP-solution gave the second largest increase in proline concentration, after IAA, as compared with the control.

BADANOVA and LEVINA (1970) established that after spraying barley plants with 0.01 per cent GA_3-solution the thermo- and drought-tolerance of plants decreased. In our experiments proline-accumulation was promoted by GA_3 but in a lesser degree.

At any rate, from our results the conclusion may be drawn that from among all the hormones the IAA-treatment was the most effective for raising both the level of the total amino-acid and proline increase, and that of the water balance of shoots. It is probable that this effect of the hormones is indirect and its mechanism is complicated that demands further studies.

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